

The University of Chicago

THE EFFECT OF LIVER EXTRACT ON
THE BLOOD PICTURE OF NORMAL RATS
FROM BIRTH TO TWENTY-FOUR
DAYS OF AGE

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE BIO-
LOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGY
1939

By
HARRY DAVIS BRUNER

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THE BLOOD PICTURE OF RATS FROM BIRTH TO TWENTY-FOUR DAYS OF AGE

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From the Departments of Physiology of the University of Chicago

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A survey of the literature (Addison and Richter, 1923; Briesse, 1936; Chisolm, 1911; Drabkin and Fitz-Hugh, 1934; Enzmann, 1934; Fitz-Hugh, Robson and Drabkin, 1933; Jolly, 1906, 1909; Kindred and Corey, 1930; Orten and Smith, 1934; Smith, 1932; Sure, Kik and Walker, 1929; Wigodsky and Ivy, 1938; Wills and Mehta, 1930; Wintrobe and Shumacker, 1935, 1936) describing one or more of the components of the blood picture of rats from birth to twenty-four days of age indicated the inadequacy of data for the purpose of evaluating statistically the effects of experimental procedures on such animals. Moreover, the not inconsiderable differences between the reported values suggest the possibility of technical and colony differences. It is the purpose of this report to present a controlled set of daily observations on the blood picture of rats of the above age group, with emphasis on the determination of the degree of variability, thereby delineating statistically significant values.

MATERIALS AND PROCEDURE. The parents of the young rats were of the University of Chicago pied-albino stock, and weighed 250 or more grams. They were kept in breeding cages until near parturition and then isolated. The young remained in litter groups until used. One-half of the mothers received, *ad libitum*, a diet of boiled beef lung-bread-milk and a mixture of Klim and cornmeal. The other half received only Purina checkers.

The observations were made on 288 young rats, twelve for each day, from birth to twenty-four days of age. Rats 0 to 24 hours old were classed as one day old; those 24 to 48 hours old, two days old, etc. At least eight of the twelve rats for each day were from different litters. Seventy-six litters were sampled. As far as possible it was attempted to space the young of a litter over the entire twenty-four days. The young were not grouped according to sex. The data were collected over a period of eleven months. The weight and length of the young agreed with data collected by Donaldson (1924).

Blood was collected by clipping off the head and allowing the body to bleed into a dry, heparin-prepared vessel. The blood values determined

were erythrocyte count, hemoglobin estimation, volume of packed erythrocytes, reticulocyte percentage, and leucocyte count. Mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration (Wintrobe, 1932) and absolute number of reticulocytes were calculated. The erythrocyte count was the average count of 1/25 sq. mm. in each of two separately filled chambers.¹ Hemoglobin was estimated by the Newcomer method. The volume of packed erythrocytes was determined by use of Van Allen hematocrit tubes. The reticulocyte percentage was derived from a count of 1000 cells of the red cell series by the wet mount technique (Wakerlin, Bruner and Kinsman, 1936); all cells showing formed, vitally stained bodies were considered reticulocytes. The leucocyte count was the average count of 8 sq. mm. Smears were stained with Wright's stain and examined for the presence of immature cell forms.

The error of observation derived from a statistical analysis of ten determinations on a single blood sample is presented in table 1. Data derived from fifty-two counts on twenty-one normal, adult, female rats of the same strain and by the same technique are presented in table 2 for purposes of comparison.

RESULTS. The observations on the 288 young rats enumerated above are condensed and summarized in table 3. From the work sheet only the means ($\bar{X}$) and the standard deviations (σ) are presented; from these values, however, most of the usual statistical values may be derived. Of the 2,592 separate determinations comprising table 3, only two (0.08 per cent) are beyond the limits of $\bar{X} \pm 3\sigma$. The coefficients of variation of the values in table 3 are larger in 99.5 per cent of instances than the corresponding coefficients of variation in table 1.

As seen in both the wet mounts and the dry smears, the erythrocytes, particularly of the younger rats, exhibited wide variations in size. Poikilocytosis and polychromatophilia were apparent only in preparations from rats less than six days old. The high reticulocyte counts of the first four days were due to the large proportion of cells containing small, discrete, vitally staining particles. At the lower level of 20 per cent to 30 per cent the reticulocytes were the usual rod, reticulum, or flake type. Frequently the reticulum, as seen in wet mounts, was so dense as to suggest the condensed nucleus of the normoblast but immature forms were seen very infrequently in the dry smears; basophilic stippling was never observed in the dry smears.

Litter mates exhibited variations in blood picture as large as those among unrelated animals of the same age.

The possible correlations of the means in table 3, except those which

¹ Red and white cell pipettes and brightline hemacytometer were certified by the United States Bureau of Standards.

reduce to a primary set of observations or reciprocals, were tested, but were not judged significant.

By use of the method of running averages, it is found that the erythrocytes steadily increase at the rate of approximately 100,000 per c.mm. per day. The mean corpuscular volume and the mean corpuscular hemoglobin decrease at a fairly constant rate up to the eighteenth day. From the

TABLE 1

Statistical analysis of 10 determinations on one blood sample indicating error of observation

	R.B.C.*	Hb.*	Ht.*	M.C.V.*	M.C.Hb.*	M.C.Hb.C.*	RETICULO- CYTES*		W.B.C.*
							Per cent	Abso- lute	
Mean.....	2.720	7.18	25.25	92.90	26.44	28.46	16.6	0.451	3.395
Standard deviation....	0.106	0.174	00.50	4.70	1.20	0.27	1.34	0.0012	0.449
Coefficient of variation (per cent).....	3.89	2.40	1.98	5.06	4.50	0.95	8.07	0.27	3.20

* R.B.C. = Erythrocytes in millions per c.mm.; Hb. = Hemoglobin in grams per 100 cc. blood; Ht. = Per cent packed erythrocytes; M.C.V. = Mean corpuscular volume in cubic micra; M.C.Hb. = Mean corpuscular hemoglobin in gamma gamma; M.C.Hb.C. = Mean corpuscular hemoglobin concentration in per cent; Reticulocytes expressed in per cent erythrocytes and absolute in millions per c.mm.; W.B.C. = Leucocytes in thousands per c.mm.

TABLE 2

Statistical analysis of 52 counts on 21 normal adult female rats

	R.B.C.*	Hb.*	Ht.*	M.C.V.*	M.C.Hb.*	M.C.Hb.C.*	RETICULO- CYTES*		W.B.C.*
							Per cent	Abso- lute	
Mean.....	9.422	14.90	46.44	49.7	16.3	32.7	2.6	0.226	13.476
Standard deviation....	0.508	2.54	7.72	3.90	1.31	1.91	2.00	0.156	7.280
Coefficient of variation (per cent).....	5.39	17.04	16.62	7.8	8.1	5.2	76.9	69.5	54.0

* See footnote, table 1.

eighteenth to the twenty-fourth day the means of these two values (table 3) hold levels higher than the means of the corresponding adult values (table 2). The running averages of the hemoglobin and hematocrit show a decrease for the first eight days; a plateau for six days is followed by another drop; then a definite rise begins at the eighteenth day. It is noteworthy that about the eighteenth day the mothers showed a disinclination to nurse and the young became more active and began to forage. The reticulocytes

drop from a high of 88.8 per cent at birth to 30.1 per cent on the fourth day and vary between 19 per cent and 32 per cent during the remaining twenty days. Individual variations of this determination are large, as indicated by the mean coefficient of variation of 25.4 per cent. The mean absolute number of reticulocytes decreases from 2,000,000 per c.mm. to about 1,000,000 per c.mm. on the fourth day, a level which is then held with small variations. The mean corpuscular hemoglobin concentration gradually decreases from 31.5 per cent to 28 per cent during the twenty-

TABLE 3

*Means ($\bar{X}$) and standard deviations (σ) of blood values of 288 young rats
(12 rats each day, from birth to 24 days of age)*

AGE	R.B.C.*		Hb.*		Ht.*		M.C.V.*		M.C.Hb.*		M.C. Hb.C*		RETICULOCYTES*						W.B.C.*	
													Per cent		Absolute					
	$\bar{X}$	σ	$\bar{X}$	σ	$\bar{X}$	σ	$\bar{X}$	σ	$\bar{X}$	σ	$\bar{X}$	σ	$\bar{X}$	σ	$\bar{X}$	σ				
days																				
1	2.400	0.401	10.31	1.58	32.89	5.60	134.6	10.9	43.9	3.6	31.7	2.9	88.8	3.5	2.041	0.354	4.65	2.28		
2	2.420	0.296	10.06	1.33	32.13	3.48	132.9	10.8	41.8	5.1	31.5	2.2	79.4	9.6	1.934	0.436	3.95	1.44		
3	2.560	0.349	10.39	1.43	33.12	3.43	130.5	9.8	39.9	3.7	31.3	1.8	49.3	22.6	1.264	0.631	4.10	2.46		
4	2.720	0.477	10.72	2.06	32.56	4.45	120.7	9.8	38.4	4.2	31.8	3.0	33.2	17.2	0.915	0.551	4.00	1.51		
5	2.640	0.325	9.92	1.41	32.00	3.97	121.2	12.5	37.7	4.4	31.5	1.3	30.1	7.6	0.804	0.179	3.65	1.09		
6	2.710	0.525	9.21	1.74	30.31	4.15	113.8	15.6	34.6	4.8	30.4	1.9	23.4	5.3	0.639	0.202	4.50	2.92		
7	2.685	0.351	8.74	0.75	29.21	1.70	111.0	12.3	33.3	5.4	29.9	2.0	29.0	6.8	0.785	0.241	3.75	1.40		
8	2.890	0.551	8.39	0.73	28.77	2.48	101.4	11.5	29.5	3.8	29.2	1.8	33.2	10.8	0.943	0.291	3.80	1.17		
9	3.190	0.541	8.25	0.99	27.60	2.68	88.6	13.9	26.2	3.8	29.8	2.0	28.8	12.0	0.909	0.275	3.70	0.59		
10	3.070	0.627	8.01	0.91	26.92	3.34	89.2	9.4	26.6	3.0	29.5	1.8	29.4	7.5	0.869	0.180	3.90	1.06		
11	3.385	0.548	8.31	1.07	28.90	4.69	85.2	7.2	24.9	1.6	29.0	1.4	31.6	7.6	1.095	0.357	3.30	1.13		
12	3.125	0.393	8.01	0.99	26.60	3.79	85.7	8.2	25.7	2.5	30.1	2.0	33.2	10.1	1.047	0.365	4.00	1.35		
13	3.410	0.378	7.71	0.83	27.06	3.12	79.5	6.0	22.7	1.6	28.6	2.0	31.0	5.5	1.053	0.198	3.75	1.11		
14	3.840	0.597	8.47	0.81	28.04	2.49	73.4	6.0	22.2	1.7	30.2	1.2	23.5	6.3	0.900	0.281	4.85	0.60		
15	3.800	0.517	8.20	1.09	26.79	3.40	70.9	6.5	22.5	1.7	30.7	1.8	24.2	5.4	0.897	0.185	4.55	1.13		
16	3.565	0.588	7.43	0.86	24.79	4.07	68.8	3.7	21.0	1.6	30.3	2.3	25.1	8.6	0.844	0.200	4.35	0.78		
17	3.980	0.527	7.14	0.68	24.54	2.91	62.0	5.3	18.1	1.7	30.1	2.1	21.7	4.9	0.827	0.220	4.65	1.31		
18	3.845	0.595	7.23	1.08	23.90	3.55	62.5	5.9	18.9	2.2	30.3	1.8	21.1	5.6	0.799	0.209	3.95	0.99		
19	4.470	0.377	8.04	1.37	23.42	5.25	59.7	5.4	17.9	2.1	29.9	1.5	21.5	5.2	0.909	0.197	4.85	1.05		
20	4.245	0.459	7.54	0.62	25.71	1.97	60.8	5.1	17.9	2.1	29.3	1.9	18.8	3.7	0.794	0.154	4.35	1.26		
21	4.580	0.564	8.03	1.02	27.69	3.70	60.6	5.2	18.0	2.2	29.1	1.7	22.7	4.7	1.034	0.273	5.15	1.52		
22	4.390	0.603	7.45	1.19	26.44	3.56	60.6	6.1	17.0	1.9	28.0	1.3	23.6	5.9	1.057	0.289	4.85	1.38		
23	4.675	0.417	8.17	0.95	28.88	3.89	62.1	7.3	17.6	2.4	28.3	1.3	26.8	4.7	1.253	0.257	3.85	0.82		
24	5.040	0.727	8.52	1.25	30.13	5.05	59.8	4.8	17.0	1.4	28.4	1.1	23.2	5.6	1.166	0.351	5.45	1.69		

* See footnote, table 1.

four day period. The leucocytes slowly increase by approximately 1,000 per c.mm. during the period of observation.

By use of the standard error of two means method, it was found that the differences between the corresponding mean values of adults (table 2) and young at 24 days (table 3) were not attributable to chance, except in the instance of the mean corpuscular hemoglobin. Although the ranges of the mean corpuscular volumes of adult and young overlap, the difference between the means is real.

No significant differences were observed in the blood pictures of the young of mothers fed the two different diets.

DISCUSSION. The degree of variability or scatter, as expressed by the standard deviation from the mean, is proportionally large and markedly broadens the limit of non-significance ($\bar{X} \pm 3 \sigma$). In most instances the wide boundaries of non-significance on one day include the means of ten days before or after. It will be observed that only a small part of the variability may be ascribed to technique. Inasmuch as the young do not grow at the same rate, it is to be expected that blood formation in the different individuals will proceed at different rates. Chisolm (1911) has published data showing that in growing rats, from eight to twenty-one days of age, the blood volume increase lags behind the body weight increase. The data presented here show that the per cent of erythrocytes in the blood during this period is decreasing while the number of erythrocytes per cubic millimeter is steadily increasing. Thus, it is not difficult to understand how slight variations of erythropoietic rate in individual animals may result in marked variations from the calculated mean. A larger daily population would have minimized the calculated degree of variation.

Because of these considerations, only means, which are not too sharply delineated, and trends may safely be recognized. The trends of the data presented agree with those of the previously cited reports. A few of the published means for a given age agree with the means of table 3; the remainder, however, usually fall within the calculated non-significant zone. Probable reasons for these differences are not apparent, except in the instance of hemoglobin. Mitchell (1932) has concluded that the diet and parity of the pregnant mother rat may affect the hemoglobin per 100 cc. of blood of the young. Sure and Kik (1929) have shown that the diet of the lactating mother may affect the rate of hemoglobin production in the young. Drabkin and Fitz-Hugh (1934) suggest that the changes in the blood picture from birth to maturity may be related to the nutritional background and they call attention to "the existence of physiological differences" in the rat. As was earlier stated, no differences were observed in the blood pictures of the young of mothers on two different diets. While the diet may account for some of the discrepancies between the means in the literature and those presented here, the possibility of peculiar colony differences should not be discarded.

Wintrobe and Shumacker (1935, 1936) and Smith (1932) have pointed out the essential similarity between the blood picture of fetal and infant rats and the blood picture of pernicious anemia in man. Wintrobe and Shumacker (1935) further have shown the resemblances in the blood picture changes toward adult normal levels as observed in rat fetuses and newborn, and in man with macrocytic hyperchromic anemia after adequate liver therapy. The data presented here confirm these comparisons and,

in addition, point out that the gradually decreasing mean corpuscular hemoglobin concentration is like that observed during induced remission of pernicious anemia (Wintrobe, 1931). A dissimilarity, however, is observed in the behavior of the reticulocytes which remain at a high level despite the fact that the erythrocytes per cubic millimeter are constantly increasing.

According to one cytologic view, as concisely expressed by Jones (1938) and by Kato (1935), megaloblastic erythropoiesis is the normal process in the fetus and in man with pernicious anemia, whereas normoblastic erythropoiesis is the normal process in the normal adult. The transition from megaloblastic erythropoiesis of the newborn rat to normoblastic erythropoiesis of the adult rat is reflected in the data presented, particularly in the mean corpuscular volume and the mean corpuscular hemoglobin. Analysis of the curves of these two corpuscular values and of the reticulocytes strongly suggests the presence of a "degeneration" process. The data of table 3 are interpreted as representing the composite of the decline of one type of erythropoiesis and the substitution of another. This interpretation is wholly consistent with the high degree of variation previously noted. Studies of the blood of fetal rats (Kindred and Corey, 1930, 1931; Wintrobe and Shumacker, 1935, 1936) indicate that the substitution probably begins before birth. This study indicates that the substitution is cytologically complete or nearly complete by the eighteenth day after birth, although the mean corpuscular volume after that time remains at a mean level (for six days at least) significantly above the adult normal. This inconsistency may be explained by the report of Wintrobe (1934) that the presence of a reticulocytosis raises the mean corpuscular volume. Further support for this view lies in the observation (Smith, 1932) that the reticulocytes and the corpuscular size decline simultaneously to adult levels. An alternate explanation is that sufficient megalocytes survive for a time in the circulating blood to raise the mean volume of the red cell (Jones, 1938). Numerically, the adult blood values are not attained until after the fiftieth or sixtieth day of age, as is shown by other studies of some of these values (Drabkin and Fitz-Hugh, 1934; Fitz-Hugh, Robson and Drabkin, 1933; Orten and Smith, 1934; Sure, Kik and Walker, 1929; Williamson and Ets, 1926; Wintrobe and Shumacker, 1935, 1936).

SUMMARY

1. The blood values of 288 young rats, observed in groups of twelve for each day from birth to twenty-four days of age, are recorded.
2. Observations carried out on the bloods were erythrocyte count, hemoglobin, per cent packed erythrocytes, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, reticulocytes (per cent and absolute), and leucocyte count.

3. The marked variability from the mean of these blood values is noted.
4. The essential similarity in the blood pictures of the young rat and of pernicious anemia is emphasized.
5. The changes occurring in rats' blood during the period of observation, are interpreted to be the result of transition from megaloblastic to normoblastic erythropoiesis.

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Effect of Parenteral Liver Extract on the Blood Picture of
New Born Rats.*

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Previous work^{1, 2, 3} has demonstrated the essential similarity in the transition toward normal adult levels of the peripheral blood pictures of new born rats and of the human with macrocytic hyperchromic anemia treated with liver extract. The transition in both may be interpreted^{3, 4, 5} as the result of the substitution of normoblastic erythropoiesis in the place of megaloblastic erythropoiesis. There is, therefore, the possibility that the administration of liver extract may accelerate this transition in new born rats.

A control series of observations on the blood picture changes in 288 normal rats from birth to 24 days of age has been compiled to serve as a basis of evaluation of the effect of such an experimental procedure (see Table 3, reference 3).

The degree of variability or scatter as expressed by the standard deviation is proportionally large and markedly broadens the limits of non-significance ($X \pm 3\sigma$). Inasmuch as the young do not grow at the same rate, it is to be expected that blood formation in the different individuals will proceed at different rates. Litter mates frequently showed as large variations and differences as among unrelated animals of the same age. Data on the state of hemoconcentration in these rats are not available. A larger daily population would have smoothed the curves and reduced the variations.

* Acknowledgment is made of the generous advice and aid rendered by Dr. A. J. Carlson and Dr. G. E. Wakerlin.

¹ Wintrobe, M. M., and Shumacher, H. B., *J. Clin. Invest.*, 1935, **14**, 837.

² Smith, C., *J. Path. and Bact.*, 1932, **35**, 717.

³ Bruner, H. D., Van de Erve, J., and Carlson, A. J., *Am. J. Physiol.*, 1938, **124**, 620.

⁴ Jones, O. P., *Proc. Soc. Exp. Biol. and Med.*, 1938, **38**, 222.

⁵ Kato, K., *J. Lab. and Clin. Med.*, 1935, **20**, 1243.

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Because of these considerations it is emphasized that only means, which are not too sharply delineated, and trends may safely be recognized. A sample of the error of observation is indicated by the coefficients of variation derived from 10 determinations on one blood specimen (see Table I, reference 3).

Two approaches have been utilized in determining the effect of liver extract on the blood picture of young rats: (I) The injection of liver extract into the mother rats before and during pregnancy, with examination of the blood of the young at intervals after birth. (II) The injection of liver extract directly into the young after birth, with examination of the blood at intervals after injection.

The liver extract used was a clinically active commercial extract (several different batches) formerly designated as 3 cc derived from 100 g of liver, and now as 10 units per 3 cc.† The rats used were of the same stock and fed the same diets as the control rats; further, most of the observations were carried out during the same period. The technical and hematological methods were as previously described³ and frequent checks were made to assure that the error of observation remained within fixed limits.

(I) Liver extract in doses of 2.5 cc per kg body weight was injected intramuscularly into 19 female rats once per week for 1 to 4 weeks before mating and throughout pregnancy. This weekly quantity of extract was estimated, on a weight basis, to be approximately 10 to 15 times the total quantity required to induce remission in the average case of pernicious anemia.

The blood values of the 144 young of these injected rats were determined in 32 rats within 24 hours after birth and in 14 young on the third day after birth and every third day thereafter up to the twenty-fourth day of age. The young of a litter were distributed over the entire period as far as possible.

The means and the standard deviations (Table I) of the blood values grouped as to age were checked against the control data by the method of the standard error of the difference of two means. The mean values of the erythrocytes, hemoglobin, and hematocrit were not significantly different from the corresponding means except in the instance of the erythrocytes on the twelfth, fifteenth, and eighteenth days; the means of the hemoglobin and hematocrit also tended to be higher on these days. In view of the definite non-significance on the ninth and twenty-first days, the differences do not assume importance. The increased values might readily be explained by hemoconcentration. The mean values of the mean corpuscular vol-

† Supplied in part by courtesy of Dr. Guy W. Clark, Lederle Laboratories, Inc., Pearl River, N. Y.

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TABLE I.
Means (X) and Standard Deviations (σ) of Blood Values of 144 Young Rats from Liver Extract Injected Mothers.

Age in Days	N*	Reticulocytes*											
		R.B.C.*		Hb.*		Ht.*		M.C.V.*		M.C.Hb.*		M.C.Hb.C.*	
		X	σ	X	σ	X	σ	X	σ	X	σ	X	σ
1	32	2.560	0.354	11.56	1.50	36.49	5.92	143.9	14.7	45.8	5.7	31.7	2.3
3	14	2.525	0.460	9.29	0.71	31.12	3.00	124.0	10.7	37.2	4.3	30.6	3.2
6	14	2.530	0.254	8.20	0.40	28.79	1.14	114.5	9.2	32.7	2.6	28.5	1.5
9	14	3.175	0.320	8.93	0.81	30.71	2.78	97.3	5.4	28.2	3.2	29.0	2.3
12	14	3.745	0.342	8.72	0.81	29.73	2.63	80.0	5.8	23.4	2.0	29.3	1.8
15	14	4.315	0.411	8.92	0.76	30.00	3.05	69.9	5.7	20.8	1.3	29.7	1.5
18	14	4.575	0.597	8.41	1.10	27.70	4.31	61.4	7.1	18.5	3.2	30.5	1.7
21	14	4.715	0.565	8.26	0.83	26.68	2.32	57.2	5.2	17.7	2.2	31.0	1.4
24	14	5.140	0.425	9.36	1.27	31.79	4.56	62.0	6.8	18.2	2.2	29.5	1.6

*N = Number of rats; R.B.C. = Erythrocytes in millions per mm³; Hb. = Hemoglobin in g per 100 cc blood; Ht. = % packed erythrocytes; M.C.V. = Mean corpuscular volume in cubic micra; M.C.Hb. = Mean corpuscular hemoglobin in gamma gamma; M.C.Hb.C. = Mean corpuscular hemoglobin concentration in %; Reticulocytes expressed in % erythrocytes and absolute in millions per mm³; W.B.C. = Leucocytes in thousands per mm³.

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ume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration were not significantly different. The mean values of the reticulocytes, percent and absolute, were significantly different from the means of the control groups only on the sixth day. It will be noted that the control value for this day is out of line. The leucocyte values showed no significant differences. Only 1.2% of the 1,306 measurements were beyond the limits of the control group; 0.8% of the measurements were also located beyond the limit of the $X \pm 3\sigma$ for the injected series.

There was found, thus, a lack of consistent differences between the examined blood values of the experimental and control groups. While it is possible that some of the young of the injected mothers showed significant differences, the majority did not. Further, rather large differences were sometimes obtained between similar blood values of litter mates of the same age as in the control group.

It may be added that the injection of liver extract did not prevent the appearance, qualitatively, of the prepartum anemia of the mother rat as described by Mitchell and Miller⁶ and by Van Donk, Feldman and Steenbock.⁷

(II) The liver extract used for injecting the young rats was diluted 5 times with distilled water and injected subcutaneously. Leakage was prevented by sealing the puncture with a stiff vaseline. The dose used was 5 cc per kg body weight of undiluted extract and was calculated to be approximately 20 to 25 times the remission inducing dose for the human pernicious anemia patient. A sterile saline solution containing 0.5% phenol was used for control injections.

Twenty-eight litters each of 8 or more young were used. For each 2 young rats injected with diluted liver extract, 1 litter mate was injected with salt solution to serve as control. Two litters were injected within 8 hours after birth, and similarly 2 litter groups were injected for each day after birth up to 14 days of age. Studies were made on 2 liver extract-injected rats and 1 control rat of the litter on the fourth, tenth, fifteenth and, if the litter were large enough, twenty-first days after injection, recording the blood values according to age. Of the 182 liver extract-injected young, 30 were examined after the twenty-fourth day and are not included. Since the values of the young of one age at the different intervals after injection are distributed indiscriminately among one another, the means and standard deviations of the whole distribution are presented in Table II.

⁶ Mitchell, H. S., and Miller, L., *Am. J. Physiol.*, 1931, **98**, 311.

⁷ Van Donk, E. C., Feldman, H., and Steenbock, H., *Am. J. Physiol.*, 1934, **107**, 1934.

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TABLE II.
Means (X) and Standard Deviations (σ) of Blood Values of 152 Liver Extract Injected Young Rats.

Means (X) and Standard Deviations (σ) of Blood Values of 192 Liver Extract Injected Rats																			
Age in Days	N*	Reticulocytes*																	
		R.B.C.*		Hb.*		Ht.*		M.C.V.*		M.C.Hb.*		M.C.Hb.C.*		%		Absolute		W.B.C.*	
		X	σ	X	σ	X	σ	X	σ	X	σ	X	σ	X	σ	X	σ	X	σ
4	4	2.815	0.299	8.60	.25	30.00	0.36	107.8	12.3	30.9	3.9	28.7	1.1	33.3	4.4	0.940	.173	2.15	0.42
5	4	2.405	0.250	8.34	.33	27.69	0.39	116.4	12.4	35.1	4.1	30.2	0.6	29.5	2.7	0.955	.422	3.15	1.52
6	3	3.415	0.372	8.34	.14	28.83	0.78	89.5	7.3	25.0	2.7	29.7	1.5	30.0	3.1	0.982	.164	4.12	1.65
7	4	2.895	0.501	8.64	.25	30.25	1.64	107.5	7.6	30.7	5.3	28.6	1.2	24.4	2.7	0.700	.100	3.11	0.33
8	4	2.900	0.126	8.31	.23	28.88	0.85	99.3	3.9	28.7	1.3	29.0	1.1	31.0	11.4	0.895	.310	3.64	0.89
9	4	3.490	0.465	9.06	.76	30.78	0.85	89.5	9.9	26.3	1.9	29.5	2.3	31.9	5.0	1.131	.280	3.69	0.44
10	8	3.710	0.113	8.90	.35	30.50	1.02	82.4	3.4	24.6	1.3	29.4	0.9	33.8	7.9	1.252	.275	3.63	0.75
11	11	3.445	0.177	7.99	.49	27.93	1.11	81.4	3.2	23.2	1.7	28.6	1.3	26.8	3.6	0.916	.184	3.61	0.86
12	8	3.585	0.150	7.61	.56	25.78	2.87	72.3	8.8	27.1	3.4	29.5	1.8	26.9	5.9	0.968	.152	4.28	0.81
13	5	4.135	0.122	8.46	.50	28.50	0.87	69.1	3.8	20.5	1.0	29.7	0.8	16.6	2.9	0.682	.141	4.81	0.46
14	10	4.485	0.421	9.19	.86	31.05	3.15	69.3	4.0	20.6	1.2	29.6	1.5	21.8	4.6	0.971	.201	4.40	1.99
15	12	4.000	0.403	7.78	.50	26.64	1.53	67.4	4.4	19.5	1.2	29.2	1.0	19.5	3.0	0.769	.121	3.58	0.89
16	12	3.855	0.374	7.73	.48	25.79	1.86	67.6	4.9	20.2	1.0	29.9	1.3	15.0	3.6	0.570	.157	4.44	0.89
17	15	4.230	0.197	8.08	.67	26.37	2.81	62.5	7.5	19.0	1.7	31.0	2.2	19.7	5.9	0.838	.244	3.56	0.69
18	6	4.230	0.155	7.22	.33	23.59	0.85	55.8	4.7	17.1	1.6	30.7	1.3	21.0	1.7	0.889	.088	4.14	1.13
19	11	4.185	0.332	7.13	.54	24.39	1.48	58.5	1.3	17.3	2.0	29.3	1.6	17.9	3.7	0.750	.131	5.48	1.53
20	4	4.875	0.351	8.74	2.24	28.00	5.22	67.0	7.1	17.7	3.5	30.8	2.2	22.2	8.8	1.053	.351	4.28	1.49
21	6	4.600	0.196	8.03	.18	28.21	1.80	57.0	2.9	17.5	0.9	29.5	1.4	23.6	2.7	1.076	.051	4.61	0.49
22	12	4.905	0.531	7.91	.59	27.40	3.07	56.0	5.4	16.2	2.9	29.0	1.7	26.3	6.8	1.180	.328	3.40	0.78
23	9	5.595	0.714	9.06	.49	32.08	1.73	58.1	5.3	16.4	4.0	28.2	0.7	22.9	5.7	1.313	.479	4.39	1.85
24	4	5.415	0.806	9.04	.78	32.31	2.77	61.6	14.1	17.2	4.0	28.0	1.1	22.8	2.8	1.223	.148	4.75	1.47

*R.B.C. = Erythrocytes in millions per mm³; Hb. = hemoglobin in g per 100 cc blood; Ht. = % packed erythrocytes; M.C.V. = Mean corpuscular volume in cubic micra; M.C.Hb. = Mean corpuscular hemoglobin in gamma gamma; M.C.Hb.C. = Mean corpuscular hemoglobin concentration in %; Reticulocytes expressed in % erythrocytes and absolute in millions per mm³ W.B.C. = Leucocytes in thousands per mm³; N = Number of rats.

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The means of the erythrocyte values of the injected rats from the twelfth to the eighteenth days tended to be higher than the means of the controls but the difference was not significant nor was the inflection maintained. A few isolated means of the other 8 blood values were statistically significant; the remainder were not. Thus, the great majority of the variations observed could have occurred by chance.

Comparison of the values of the 2 liver-injected rats with their litter mate control showed that the control value was located between the 2 corresponding values of the injected rats in 41%, 39%, and 42% of instances on the fourth, tenth and fifteenth days after injection respectively. This is approximately what should occur by chance. The blood values of the 30 injected rats between the twenty-fifth and thirtieth days continued the trends of the control group and were comparable to the litter control rats. Of the total of 1,368 determinations, only 0.95% were beyond the limits of $X \pm 3\sigma$ of the control group.

Figures 1 and 2 present graphically the most useful criterion, the mean corpuscular volumes of the erythrocytes, in estimating the transition changes in the blood of the experimental groups as compared to the control group of young rats. None of the means are statistically significant.

Growth in both experimental groups of rats as judged by the

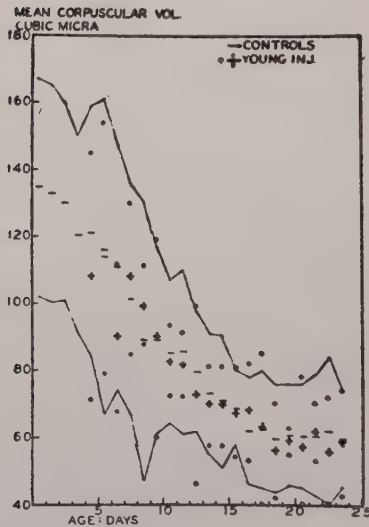


FIG. 1.

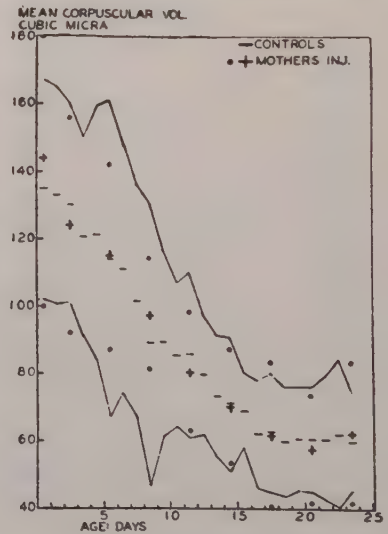


FIG. 2.

Figs. 1 and 2 illustrate the location of the means (+) and the $\pm 3\sigma$ (•) of the mean corpuscular volumes of the erythrocytes of the 2 experimental groups of young rats at indicated ages after birth in relation to the mean (—) $\pm 3\sigma$ (continuous line) of the control rats.

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weight and crown-tailbase length was comparable to control data.

The lack of a marked acceleration of normoblastic erythropoiesis or of a lessened megaloblastic erythropoiesis is indicated especially by the not significantly different decline of the mean corpuscular volume, level of the reticulocytes, and increase of erythrocytes. Fitz-Hugh, Creskoff and Taylor⁸ indicated the ineffectiveness of daily injections of liver extract in changing the reticulocyte count of 14 nursling rats. The injection of liver extract into the fetuses of 2 pregnant rats was likewise ineffective. Wintrobe, *et al.*,⁹ found that liver extract injected into 3 pregnant rabbits produced no effect on the erythrocytes of fetuses extracted before term. Also, the injection of 0.15 cc of liver extract into the placentas of rabbit fetuses in one horn of the uterus did not alter the blood picture of those fetuses in comparison with the fetuses in the uninjected horn. These workers reported that extracts of fetal pig livers and of the placenta, even in the last third of pregnancy, were inactive. Wigodsky, *et al.*,¹⁰ however, found fetal calf livers anti-anemically active. Wigodsky and Ivy¹¹ injected liver extract into pregnant rats from 2 to 14 days before delivery. They observed no significant differences between the values of the erythrocytes, hemoglobin, hematocrit, mean corpuscular volume, or the mean red cell diameter of new born rats from normal and antenatally injected mothers. Their data indicate variations similar to those encountered above.

Briese and Higgins¹² have reported that the addition of ventriculin to the diet of pregnant rats resulted in a decrease of the mean diameter of the erythrocytes of the new born rats together with a shift of the Price-Jones curve to the left as measured from dry smears. The variability in the erythrocyte counts of normal new born led them to suggest that variations in the maternal stores of the anti-anemic principle may occur. Stasney and Higgins¹³ have reported that the daily intraperitoneal injection of neutralized normal human gastric juice into pregnant rats for 12 days preceding parturition resulted in a reduction of the diameters and corpuscular volumes of the erythrocytes of the new born. Heated gastric juice

⁸ Fitz-Hugh, T., Jr., Creskoff, A. J., and Taylor, H. B., *J. Clin. Invest.*, Proc., 1936, **15**, 468.

⁹ Wintrobe, M. M., Kinsey, R. E., Blount, R. C., and Trager, W., *Am. J. Med. Sci.*, 1937, **193**, 449.

¹⁰ Wigodsky, H. S., Richter, O., and Ivy, A. C., *Am. J. Physiol.*, Proc., 1938, **123**, 215.

¹¹ Wigodsky, H. S., and Ivy, A. C., *Proc. Soc. Exp. Biol. and Med.*, 1938, **38**, 787.

¹² Briese, E., and Higgins, G. M., *Anat. Rec.*, 1939, **73**, 105.

¹³ Stasney, J., and Higgins, G. M., *Proc. Staff Meetings Mayo Clinic*, 1937, **12**, 490.

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produced no effect. Similar effects were obtained by Stasney, Higgins, and Mann¹⁴ using the gastric juice obtained from a fundic pouch of a pig. Briese¹⁵ reported that the blood picture of the new born of rats from mothers rendered anemic by daily inhalations of CCl_4 during pregnancy was more macrocytic than the blood of young from normal mothers.

The lack of significant changes probably are not due to administration of insufficient amounts nor to the mode of administration of liver extract. Connery and Goldwater¹⁶ and Riddle and Sturgis¹⁷ have shown that single massive doses are effective in inducing remission in human pernicious anemia. It is interesting to observe also that the liver preparation did not produce a reduction in erythrocytes or hemoglobin. This effect of liver has been reported by Marshall¹⁸ after injection of liver into rats made polycythemic by cobalt. Adlersberg and Leiner¹⁹ reported that anemia may be produced in normal rabbits by the administration of liver preparations both orally and parenterally; a powdered liver preparation given orally to dogs also produced an anemia.

Assuming that the rat requires the anti-pernicious anemia principle, the lack of effect of the administered liver extract in the young rats is similar to that observed in the human macrocytic hyperchromic anemia patient previously given optimal amounts of liver extract. It would appear that in the presence of adequate amounts of anti-anemia substance some other factor becomes the limiting agent in the mechanism of blood production.

Summary. 1. The transition from megaloblastic to normoblastic erythropoiesis appears to be reflected in data on the blood cell values of normal rats from birth to 24 days of age. 2. Blood cell values of young whose mothers were injected with liver extract before and during pregnancy were found to be not significantly different from control values at comparable ages after birth. 3. Blood cell values of young rats directly injected with liver extract up to the fourteenth day after birth were found not to be significantly different from control values at comparable ages after birth. 4. The young rat's reaction to the presence of excess anti-anemic principle resembles that of the human macrocytic hyperchromic anemia patient maintained on adequate amounts of liver extract.

¹⁴ Stasney, J., Higgins, G. M., and Mann, F. C., *Proc. Staff Meetings Mayo Clinic*, 1937, **12**, 699.

¹⁵ Briese, E., *Am. J. Med. Sci.*, 1938, **195**, 787.

¹⁶ Connery, J. E., and Goldwater, L. J., *J. Lab. and Clin. Med.*, 1932, **17**, 1016.

¹⁷ Riddle, M. C., and Sturgis, C. C., *Am. J. Med. Sci.*, 1930, **180**, 1.

¹⁸ Marshall, L. H., *Am. J. Physiol.*, 1936, **114**, 194.

¹⁹ Adlersberg, D., and Leiner, G., *Z. f. d. ges. exper. Med.*, 1936, **98**, 398.

